JASEM ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://ww.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 22 (3) 379 – 385, March 2018

Efficacy of the stem powder of an invasive alien plant, *Chromolaena odorata* (L) (Asteraceae) against *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae)

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ABSTRACT: This study investigated the repellent and insecticidal activities of the stem powder of *C. odorata* against a serious economic pest of cowpea, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). Cowpea grains infested with ten (10) 1 – 2 day old unsexed adults of *C. maculatus* were exposed to three concentrations (0.0, 2.6 and 5.0 g) of *C. odorata* stem powder after which percentage repellency was monitored for 12, 24, 36 and 48 hours and mortality for 12, 24, 36, 48 and 72 hours. Results from this study revealed that the stem powder of *C. odorata* exhibited significant repellent activity against *C. maculatus*, although it was a function of both concentration and exposure duration. Following a 48 – hour exposure period of *C. maculatus* to the stem powder of *C. odorata,* the highest concentration (5.0 g) was observed to demonstrate the highest percentage repellent activity (87%). Similarly, mortality of *C. maculatus* caused by the stem powder of *C. odorata* was high and observed to be concentration and exposure time dependent. At the lowest concentration (2.6 g), the stem powder of *C. odorata* accounted for 100% mortality of *C. maculatus* after a 72 – hour exposure period. In summary, this present study clearly demonstrates the repellent and insecticidal activities of *C. odorata* stem powder and further suggests its usage as an attractive alternative to synthetic insecticides in the management of *C. maculatus* infestation in Nigeria and elsewhere.

DOI: https://dx.doi.org/10.4314/jasem.v22i3.15

Dates: Received: 21 February 2018; Revised: 15 March: 2018; Accepted: 30 March 2018

Keywords: Cowpea, *Chromolaena odorata*, stem powder, toxicity, Mortality, repellency, *Callosobruchus maculatus*

Cowpea, *Vigna unguiculata* L. (Walp) is one of the most economically important crops grown in the tropical and subtropical regions of the world (Uddin and Abdulazeez, 2013). Further, it is the most important grain in the farming systems of Nigeria and West Africa as a whole (Singh *et al*., 2002). Nigeria being the largest producer and consumer of cowpea, accounts for 61% and 58% of production in Africa and in the world respectively (FAO, 2012). Cowpea is known to act as a cheap dietary source of protein, where it accounts for 60% of protein intake in Nigeria (Ojo *et al*., 2013) and complement to cereal diets (Phillips *et al*., 2003). Cowpea is also a rich source of calories, minerals and vitamins (Akinkurolere, 2012). Despite all of these, its continuous production and storage have been under severe threats by insect pests (Akinkurolere, 2012).

Callosobruchus maculatus (Coleoptera: Chrysomelidae) is a serious field-to-store pest of cowpea which causes considerable damages (up to 100%) to stored cowpea grains when they are left unprotected (Gbaye *et al*., 2011). Further, they have been tagged as the most notorious pest of other leguminous grains such as lentils, green gram, chicken pea, blackgram, soybean and haricot beans (Park *et al*., 2003; Rahman and Talukder, 2006). Infestation by this insect pest normally begins in the field before harvest, where gravid females oviposit on ripening cowpea pods followed by the emergence of the larvae which find its way into the seeds after harvest and storage (Howe, 1965), where they feed on the cotyledon and embryo. All of these activities invariably exert grave effects on the quality and quantity of the cowpea seeds, consequently, reducing their market value, and rendering them unfit for human consumption (Akinkurolere *et al*., 2007). In addition, exit holes created on the seeds after the emergence of the adults predisposes the seeds to contamination by mycotoxins (Carvalho *et al*., 2016) and also make the seeds unsuitable for sowing purposes (Akinkurolere, 2012).

While the use of synthetic insecticides such as phosphine, deltamethrin, methyl bromide amongst others in the control of stored product pests including *C. maculatus* are not uncommon (Akinkurolere, 2012; Carvalho *et al*., 2016), existing reports however

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show that their overzealous usage have led to various unforeseen problems such as the development of resistant strains of insect pests, adverse effects on non-targeted organisms, cost and toxicity to consumers (Aboua *et al.*, 2010; Adedire *et al*., 2011). All of these drawbacks, have triggered the urgent need to search for eco-friendly alternatives that are readily available and affordable (Kedia *et al*., 2015), thus the reason for the increasing interest in the use of powders, oils and/or extracts from both indigenous and invasive alien plants in insect pest management.

One of the plants used in controlling insect pests including pests of stored products is *Chromolaena odorata* (L.) (Asteraceae), an invasive alien plant that is native to the Americas, from Northern Argentina to Southern Florida, USA, including the Caribbean Islands (Uyi *et al*., 2014). Following the accidental introduction of the weed into Nigeria in the late 1930s, it has been reported to have negative impacts on agriculture, biodiversity conservation, and human livelihoods (Uyi *et al*., 2014). Despite its negative impacts, a number of studies have empirically demonstrated its usefulness in controlling insect pests (e.g. Udebuani *et al*., 2015; Lawal *et al*., 2015; Ahad *et al*., 2016). While several studies (e.g. Uyi and Igbinoba, 2016) have demonstrated the repellent and toxicological activities of the leaf and root powders or extracts of *C. odorata* against stored product pests including *C. maculatus*, studies categorically focusing on the activities of the stem powder or extract are still scarce (but see Uyi and Obi, 2017). Although Uyi and Obi (2017) investigated the efficacy of the stem powder of *C. odorata* against *C. maculatus*, these authors only used a single concentration of the powder. Therefore, the objective of this present study is to determine the repellent and insecticidal activities of three different concentrations of the stem powder of *C. odorata* against the cowpea beetle, *C. maculatus*.

MATERIALS AND METHODS

Collection and preparation of plant powder: Fresh stems of *C. odorata* plants were collected from an open farmland at Dentistry quarters, within the vicinity of the University of Benin Teaching Hospital (UBTH), Benin City (6º39'N, 5º56'E), Nigeria. Following collection, the stems were chopped separately into pieces, washed with running water and shade dried to a constant weight. The dried stems were blended into fine powder using an electric blender (Braum Multiquick Immersion Hand Blender, B White Mixer MR 5550CA, Germany) and then preserved in an air-tight and water-proof container pending use.

Insect culture: Mass culture of the insect was done on cowpea grains (purchased from Uselu Market, Benin City, Nigeria) at an ambient temperature of 27 ± 2 °C and 80 ± 5 % RH in the Laboratory of the Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria. Ten pairs of adult beetles (1 – 3 day old) along with the food were placed in five 4 litre aerated plastic containers (with a screw top lid). Containers (with adult weevils) were kept for 7 days in the laboratory for mating and oviposition. The beetles were removed from the containers and the grains containing eggs laid by the beetles were transferred to separate (but similar) containers and allowed to hatch. Only the newly emerged F_2 generation of unsexed adult weevils were used for the trials.

Repellency test: The experiment was conducted at an ambient temperature of 25 ± 2 °C and 80 ± 5 % RH in the Laboratory of the Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria. Two different concentrations of the stem powder (2.6 and 5.0 g) of *C. odorata* were used. Prior to the repellency and mortality experiments, the cowpea seeds used in this trial were placed in a plastic container and transferred into a freezer and the container was left for 48 hours. The above procedure was employed to ensure that the grains were pest-free before using them for the test. Fifty grams of cowpea grains was placed inside a screw top plastic container (100 ml) and treated with 2.6 or 5.0 g of the stem powder. The grains and stem powders were mixed before being transferred into a perforated 200 ml plastic cup and then the top was covered with aluminium foil and tightly sealed with a rubber band. Ten 1 – 2 day old unsexed adults of *C. maculatus* were introduced into each cup through a hole made in the foil and sealed with a paper tape to prevent insects escaping. The perforated cup was placed inside a completely enclosed and transparent 2 litre plastic bucket to enable an accurate count of the beetles that exit the treated grains. The treatment was replicated ten times for each concentration (grams) and beetles were exposed for 12, 24, 36 and 48 hours. Control treatments, where the grains were not treated with *C. odorata* stem powder were also monitored for 12, 24, 36 and 48 hours. The number of insects leaving the treated grains gives a measure of repellency due to the stem powder.

Mortality bioassay: To perform the mortality bioassay, 50 g of cowpea grains was placed inside a screw top 100 ml plastic container and one of the two concentrations (2.6 or 5.0 g) of *C. odorata* stem powder was added to the grains inside the container. The grains and stem powders were mixed before being transferred into a perforated 200 ml plastic cup and then the top was covered with aluminium foil and tightly sealed with a rubber band. Ten 1 – 2 day old unsexed adults of *C. maculatus* were introduced into each cup through a hole made in the foil and sealed with a paper tape to prevent insects from escaping. The perforated cup was placed inside a completely enclosed and transparent 2 litre plastic bucket to enable an accurate count of the weevils that leaves the treated grains. The treatment was replicated ten times for each concentration. The numbers of dead beetles were monitored and counted at 12, 24, 36, 48 and 72 hours following the commencement of the experiment. No mortality was recorded 12 hours after exposure to the stem powder. Control treatments, where the grains were not treated with *C. odorata* stem powder were also monitored for 12, 24, 36, 48 and 72 hours.

Statistical Analysis: The repellent and mortality effect of two concentrations of *C. odorata* stem powders on *C. maculatus* was analysed with General Linear Model Analysis of Variance (GLM ANOVA). The effects of exposure time of the different treatment types on *C. maculatus* was analysed with Generalized Linear Model (GLZ) assuming a normal distribution with an identity link function. When the overall results were significant in the GLM analysis, the difference among the treatment means were compared using the Bonferroni's test. All data were analysed using SPSS Statistical software, version 16.0 (SPSS, Chicago, USA).

RESULTS AND DISCUSSION

The stem powder of *C. odorata* exhibited some repellent activity against *C. maculatus* compared to the control (Figure 1a-d). Following a 12 – hour exposure period of *C. maculatus* to different treatments (=concentrations) of *C. odorata* stem powder, percentage repellency significantly differed ($F_{2,27}$ = 29.29; P < .001) among treatments with the control exhibiting the least repellent activity (3%) against the beetles (Figure 1a).

Percentage repellency was higher in the grains treated with 5.0 g of *C. odorata* stem powder than the grains treated with 2.6 g (Figure 1a). Similarly, percentage repellency significantly differed ($F_{2,27}$ = 94.5; P < .001) among all the three concentrations following a 24 – hour exposure duration, with 5.0 g of *C. odorata* stem powder exhibiting the highest repellent activity (42%) against *C. maculatus* (Figure 1b). After a 36 – hour exposure interval, 2.6 and 5.0 g of *C. odorata* stem powders exhibited a significantly higher ($F_{2,27}$ = 36.55; P < .001) percentage repellency against the beetles when compared to the control treatment (Figure 1c).

However, percentage repellency significantly differed ($F_{2,27}$ = 641.83; P < .001) among all three treatments following a 48 – hour exposure period, with 5.0 g of *C. odorata* stem powder exhibiting the highest (87%) repellent activity against *C. maculatus* (Figure 1d). Overall, percentage repellency significantly increased with increased exposure time in the 2.6 and 5.0 g treatments (Figure 2, Table 1).

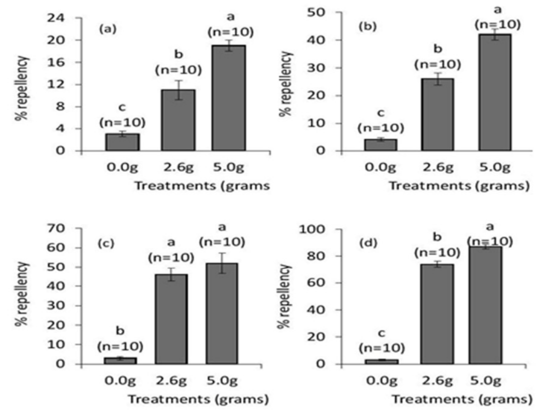

Fig 1: Percentage (mean ± se) repellency of different concentrations (treatments) of *Chromolaena odorata* stem powder against *Callosobruchus maculatus* exposed for 12 hours (a) 24 hours (b) 36 hours (c) and 48-hours (d). Means capped (following GLM) with different letters are significantly different (after Bonferroni test: P<0.05). Sample sizes are given in parenthesis. Control was not treated with stem powder.

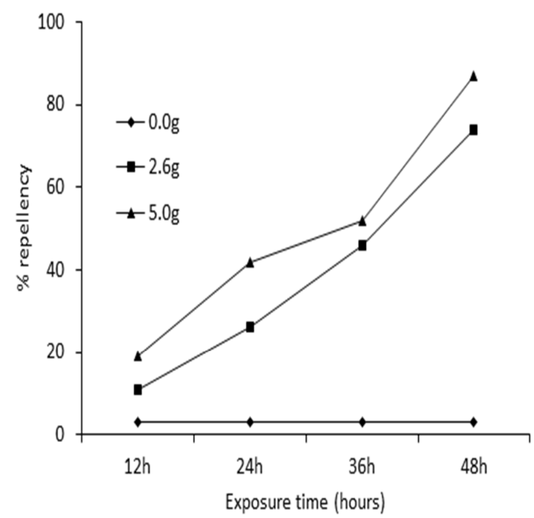

Fig 2: Percentage (mean) repellency of different concentrations (=treatments) of *Chromolaena odorata* stem powder against

Callosobruchus maculatus at different exposure periods (time: 12h, 24h, 36, 48h). Control was not treated with stem powder.

Table 1: Generalized linear model (GLZ) results for effects of different treatments of *Chromolaena odorata* stem powder, exposure time and their interactions on mortality of, and repellency against *Callosobruchus maculatus*. Following arcsine square root transformation of the data, normal distributions with an identity link function were assumed.

Effect	d.f.	Wald $\chi 2$	*P*
% Repellency			
Intercept	1	116563.33	**0.0001**
Treatment	2	45781.67	**0.0001**
Exposure time	3	30750.00	**0.0001**
Treatment x exposure time	6	15605.00	**0.0001**
% Mortality			
Intercept	1	152322.67	**0.0001**
Treatment	2	69649.33	**0.0001**
Exposure time	4	88817.33	**0.0001**
Treatment x exposure time	8	44330.67	**0.0001**

Results from this study revealed that the stem powder of *C. odorata* exhibited repellent and insecticidal activities against *C. maculatus*. In this study, percentage repellency against *C. maculatus* when exposed to cowpea grains (treated with different concentrations of *C. odorata* stem powder) was dependent on the concentration and duration of exposure. Following a 48 – hour exposure period, the highest concentration (5.0 g) of *C. odorata* stem powder demonstrated the highest repellent activity, repelling 87% of the beetle. This result conforms to those from previous studies (e.g. Udebuani *et al*., 2015; Uyi and Igbinoba, 2016) where insect repellency was observe to increase with increasing concentrations of powders and extracts from plants. As has been documented in other studies (e.g. Lawal *et al*., 2015; Uyi and Obi, 2017), percentage repellence increased with an increase in the exposure period of the pest to the stem powder of *C. odorata*. For example, when cowpea grains were treated with the root powder of *C. odorata*, the highest concentration (3.98 g) significantly repelled 91% of *C. maculatus* following a 48 – hour exposure period (Uyi and Igbinoba, 2016).

Treating *C. maculatus* infested cowpeas with the stem powder of *C. odorata* resulted in varying mortality levels in the beetles (Figure 3a-d). When cowpea beetles were exposed for a 24 – hour period to different treatments of *C. odorata* stem powder (including the control), mortality differed significantly ($F_{2,27}$ = 77.16; P < .001) with the control recording the least mortality (Figure 3a). Following a 36 – hour exposure period of the beetles to different concentrations of *C. odorata* stem powder, percentage mortality significantly differed ($F_{2,27}$ = 69.82; P < .001) with 2.6 and 5.0 g of the stem powders causing higher mortality (37% and 44% respectively) relative to the control (Figure 3b). In the 48 hours exposure trial, percentage mortality also differed significantly ($F_{2,27}$ = 585.21; P < .001) with 5.0 g of the stem powder causing the highest mortality (84%) followed by the 2.6 g treatment (65%) and then the control (2%) exhibiting the least percentage mortality (Figure 3c). Finally, in the 72 hours exposure trial, beetle mortality also differed significantly ($F_{2,27}$ = 5402.25; P < .001) among all three treatments with both 5.0 and 2.6 g of *C. odorata* stem powder accounting for 100% mortality compared to the control which only accounted for 2% mortality (Figure 3d). Overall, percentage mortality significantly increased with an increase in exposure time in all treatments except for the control (Figure 4; Table 1).

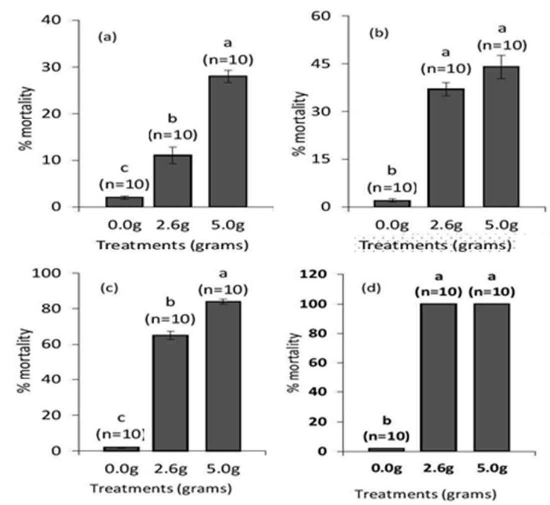

Fig 3: Percentage mortality (mean ± se) of *Callosobruchus maculatus* caused by different treatments of *Chromolaena odorata* stem powder following a 24-hour (a), 36-hour (b), 48-hour (c) and 72-hour (d) exposure period. Means capped (following GLM) with different letters are significantly different (after Bonferroni test: P<0.05). Sample sizes are given in parenthesis. Control was not treated with stem powder.

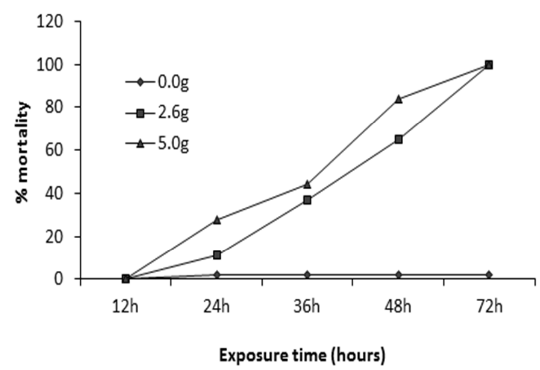

Fig 4: Percentage mortality (mean) of *Callosobruchus maculatus* caused by different concentrations of the stem powder of

Chromolaena odorata plants at different exposure periods (time: 12h, 24h, 36, and 48 h).

Admittedly, empirical evidences on the insecticidal activity of the leaf, stem and root powders or extracts of *C. odorata* against insect pests including those of stored products are not uncommon (Udebuani *et al*. 2015; Uyi and Igbinoba, 2016; Ahad *et al*. 2016), nonetheless, studies categorically focusing on the insecticidal activity of the stem powder against *C. maculatus* are still scarce. After exposing *C. maculatus* to cowpea grains treated with different concentrations of *C. odorata* stem powder for 72 hours, the highest concentration (5.0 g) exhibited the highest insecticidal activity, causing 100% mortality in *C. maculatus*. This result corroborates with the findings of other authors (e.g. Ogendo *et al*., 2008; Brisibe *et al*., 2011; Lawal *et al*., 2015; Uyi and Igbinoba, 2016) who reported high mortalities with increasing concentrations of either plant powders, oils or extracts against insect pests including those of stored products. Furthermore, our current result is superior to the findings of a previous study by Uyi and Obi (2017) who comparatively investigated the insecticidal activities of the leaf, stem and root powders of *C. odorata* against *C. maculatus*. The authors reported that cowpea grains treated with 1.96 g of *C. odorata* stem powder was only able to cause 65% mortality in *C. maculatus* at the end of the experiment. As is common with other studies (e.g. Uyi and Igbinoba; 2016; Uyi and Obi, 2017), insect mortality increased considerably with an increase in exposure time. For instance, evaluating the insecticidal efficacy of mixed leaf powders of *Vernonia amygdalina* (L.) (Asteraceae) and *Ocimum gratissimum* (Del.) (Lamiaceae) against *C. maculatus*, Musa *et al*. (2009) reported that the mixed leaf powders of both plants demonstrated remarkable insecticidal activities against *C. maculatus* after a 72 – hour exposure.

A number of explanations might exist for the results (i.e. high mortality and repellency) obtained in this study. First, plant powders are characterized by the presence of fine particles which might block the spiracles of the insects thus leading to death by suffocation (Denloye, 2010). Second, the sequence of behaviour of insect pest especially in females while ovipositing makes them prone to acquiring toxic residues from treated surfaces (Ogunwolu and Idowu, 1994). Thirdly, plants are characterized by the presence of secondary chemicals (=bioactive compounds) which have been documented to be toxic to insect pests (Ekeh *et al*., 2013; Udebuani *et al*., 2015; Ahad *et al*., 2016). Despite all of these, we consider the presence of secondary chemicals in the stem of *C. odorata* as the most plausible explanation for the observed pattern (high mortality and repellency) recorded in this study. Although, studies on the phytochemical composition of the stem of *C. odorata* are scarce, nevertheless, our hypothesis can be validated by the findings of Agaba and Fawole, (2014) and Udebuani *et al*. (2015) who evaluated the phytochemical compositions of the roots and leaves of *C. odorata* respectively. The authors reported the presence of phytochemicals such as alkaloids, phenols, flavonoids, saponins, tannins, anthraquinones and cardenolides in the leaves and roots of *C. odorata*.

Conclusion: This present study clearly demonstrates the repellent and insecticidal activities of the stem powder of *C. odorata* against *C. maculatus*. Therefore our study suggests the usage of *C. odorata* stem powder as an attractive alternative to synthetic insecticides in the management of *C. maculatus* infestation in Nigeria and elsewhere.

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